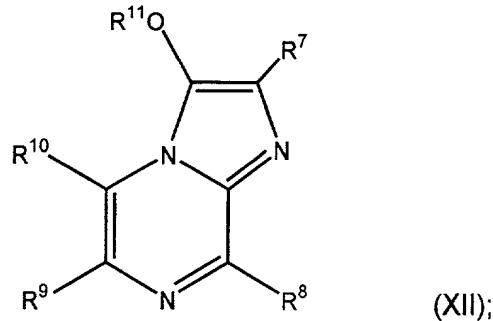


CLAIMS

- ## 1. A compound of formula (XII)



wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴.

R^8 is H, alkyl, heteroalkyl, or aryl;

R^9 is H, alkyl, heteroalkyl, aryl, or $-C_6H_4OR^{15}$.

R^{10} is -H, -CH₃, or -CH(CH₃)₂; and

R^{11} , R^{14} , and R^{15} are independently enzyme-removable groups; with the proviso that R^{11} , R^{14} , and R^{15} are not all acetyl groups.

- 10 2. The compound of claim 1, wherein
R⁷ is -CH₂-C₆H₅, naphthyl, -CH₂-C₆H₄OH, -CH₂-C₆H₄F, or -CH₂-C₆H₄OR¹⁴;
R⁸ is -CH₂C₆H₅, -CH₂C₆H₁₁, -CH₂C₅H₉, or -
(CH₂)₃NHC(=NH)NH₂; and
15 R⁹ is phenyl, indolyl, -C₆H₄OH, -C₆H₄NH₂, -C₆H₄F, or -C₆H₄OR¹⁵.

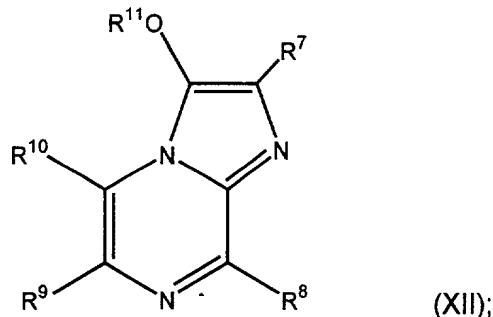
3. The compound of claim 1, wherein R¹¹, R¹⁴, and R¹⁵ are esters.

4. The compound of claim 1, wherein
R¹¹ is acetyl; and
R¹⁴ and R¹⁵ are independently butyryl, acetoxyethyl,
20 propanoyloxyethyl, butyryloxyethyl, or pivaloyloxyethyl.

5. The compound of claim 1, wherein
R¹¹ is butyryl, acetoxyethyl, propanoyloxyethyl,
butyryloxyethyl, or pivaloyloxyethyl; and

R^{14} and R^{15} are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

6. A compound of formula (XII)



wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴.

R^8 is H, alkyl, heteroalkyl, or aryl;

R^9 is H, alkyl, heteroalkyl, aryl, or $-C_6H_4OR^{15}$.

R^{10} is -H, -CH₃, or -CH(CH₃)₂; and

R^{11} , R^{14} , and R^{15} are independently enzyme-removable groups;

10 and

wherein the concentration of the compound in a mixture

comprising F12 medium and 10% fetal bovine serum at 22°C is reduced by less than 50% after 45 minutes.

7. The compound of claim 6, wherein

15 R⁷ is -CH₂-C₆H₅, naphthyl, -CH₂-C₆H₄OH, -CH₂-C₆H₄F, or -CH₂-C₆H₄OR¹⁴;

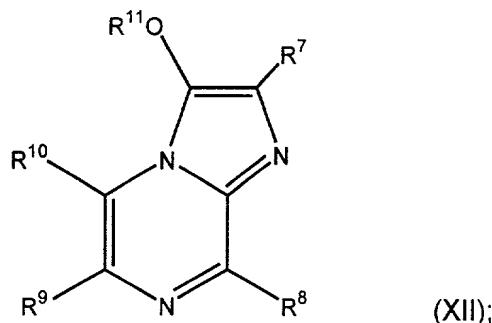
R⁸ is -CH₂C₆H₅, -CH₂C₆H₁₁, -CH₂C₅H₉, or -(CH₂)₃NHC(=NH)NH₂; and

R^9 is phenyl, indolyl, $-C_6H_4OH$, $-C_6H_4NH_2$, $-C_6H_4F$, or $-C_6H_4OR^{15}$.

20 8. The compound of claim 6, wherein R¹¹, R¹⁴, and R¹⁵ are esters.

9. The compound of claim 6, wherein R¹¹, R¹⁴, and R¹⁵ are independently acetyl, butyryl, acetoxyethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

10. A compound of formula (XII)



wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

R¹⁰ is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are independently enzyme-removable groups;

and

wherein the removal of at least one enzyme-removable group

provides a parent compound; and

wherein the time necessary for the concentration of the compound in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50% is greater than the time necessary for the concentration of the parent compound in a mixture comprising F12 medium and 10% fetal bovine serum at 22°C to be reduced by 50%.

11. The compound of claim 10, wherein the removal of at least two enzyme-removable groups provides the parent compound.

12. The compound of claim 10, wherein the removal of all enzyme-removable groups provides the parent compound.

20 13. The compound of claim 10, wherein

R⁷ is -CH₂-C₆H₅, naphthyl, -CH₂-C₆H₄OH, -CH₂-C₆H₄F, or -CH₂-C₆H₄OR¹⁴;

R⁸ is -CH₂C₆H₅, -CH₂C₆H₁₁, -CH₂C₅H₉, or -(CH₂)₃NHC(=NH)NH₂; and

R^9 is phenyl, indolyl, $-C_6H_4OH$, $-C_6H_4NH_2$, $-C_6H_4F$, or $-C_6H_4OR^{15}$.

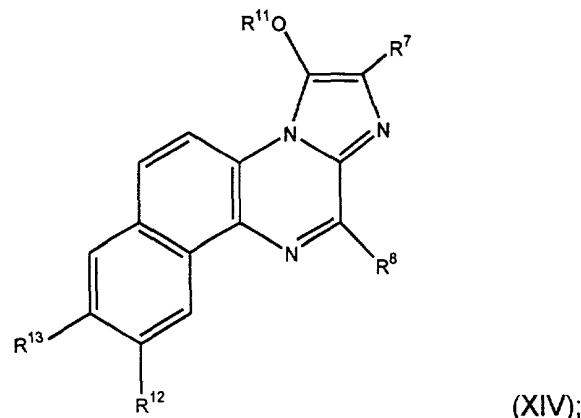
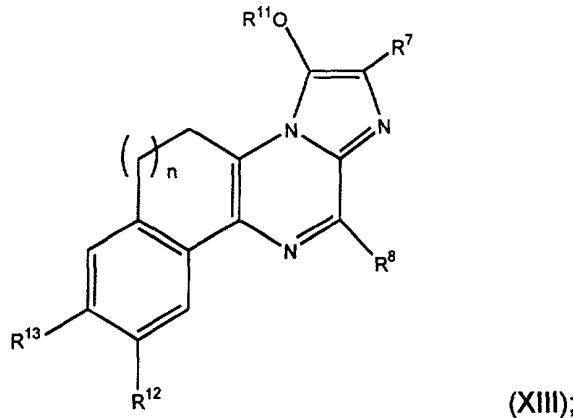
14. The compound of claim 10, wherein R^{11} , R^{14} , and R^{15} are esters.

15. The compound of claim 10, wherein R^{11} , R^{14} , and R^{15} are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl,

5

butyryloxymethyl, or pivaloyloxymethyl.

16. A compound of formula (XIII) or (XIV)



wherein R^7 is H, alkyl, heteroalkyl, aryl, or $-CH_2-C_6H_4OR^{14}$;

10 R^8 is H, alkyl, heteroalkyl, or aryl;

R^{12} and R^{13} are independently -H, -OH, alkyl, heteroalkyl, aryl,

or $-OR^{16}$;

n is 0, 1, or 2; and

R^{11} , R^{14} , and R^{16} are independently enzyme-removable groups.

17. The compound of claim 16, wherein
R⁷ is -CH₂-C₆H₅, naphthyl, -CH₂-C₆H₄OH, -CH₂-C₆H₄F, or -CH₂-C₆H₄OR¹⁴; and
R⁸ is -CH₂C₆H₅, -CH₂C₆H₁₁, -CH₂C₅H₉, or -
(CH₂)₃NHC(=NH)NH₂.
- 5 18. The compound of claim 16, wherein R¹¹, R¹⁴, and R¹⁶ are esters.
19. The compound of claim 16, wherein R¹¹, R¹⁴, and R¹⁶ are independently acetyl, butyryl, acetoxyethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.
- 10 20. The compound of claim 16, wherein n is 1.
21. A composition, comprising:
the compound of claim 1 in solution.
22. The composition of claim 21, wherein the solution is an aqueous solution.
- 15 23. The composition of claim 21, wherein the solution comprises DMSO or alcohol.
24. A composition, comprising:
the compound of claim 6, in solution.
25. The composition of claim 24, wherein the solution is an aqueous solution.
- 20 26. The composition of claim 24, wherein the solution comprises DMSO or alcohol.
27. A composition, comprising:
the compound of claim 10, in solution.

28. The composition of claim 27, wherein the solution is an aqueous solution.

29. The composition of claim 27, wherein the solution comprises DMSO or alcohol.

5 30. A composition, comprising:
 the compound of claim 16, in solution.

31. The composition of claim 30, wherein the solution is an aqueous solution.

10 32. The composition of claim 30, wherein the solution comprises DMSO or alcohol.

15 33. A protected luminophore, which is
 a modified coelenterazine;
 wherein the enol group has been converted to an ester or an
ether comprising an enzyme-removable group; the removal of said enzyme-
removable group providing a parent coelenterazine; and
 wherein the time necessary for the concentration of the modified
coelenterazine in a mixture comprising F12 medium and 10% fetal bovine
serum at 22°C to be reduced by 50% is greater than the time necessary for
the concentration of the parent coelenterazine in a mixture comprising F12
medium and 10% fetal bovine serum at 22°C to be reduced by 50%.

20

34. A kit, comprising:
 a protected luminophore; and
 a luminogenic protein.

25 35. The kit of claim 34, further comprising a deprotecting enzyme

separate from the luminophore.

36. The kit of claim 34, wherein the protected luminophore and the
luminogenic protein are in separate containers.

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37. The kit of claim 34, wherein the protected luminophore and the luminogenic protein are in the same container.

38. A kit, comprising:

a protected luminophore; and

5 a deprotecting enzyme;

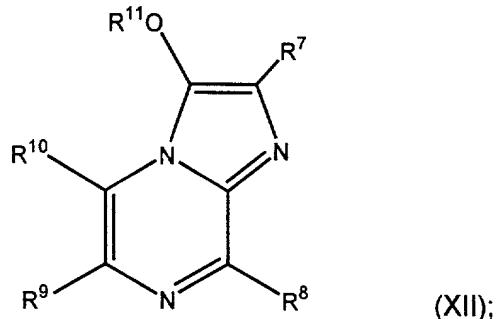
wherein the luminophore and the deprotecting enzyme are in separate containers.

39. A method of measuring the enzymatic activity of a luminogenic protein comprising:

10 contacting a luminogenic protein, a deprotecting enzyme, and a protected luminophore in solution to form a composition; and
detecting light produced from the composition.

40. The method of claim 39, wherein the luminogenic protein is *Renilla luciferase*.

15 41. The method of claim 39, wherein the protected luminophore is a compound of formula (XII)



wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

20 R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

R¹⁰ is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are independently enzyme-removable groups.

42. The method of claim 41, wherein

R⁷ is -CH₂-C₆H₅, naphthyl, -CH₂-C₆H₄OH, -CH₂-C₆H₄F, or -CH₂-C₆H₄OR¹⁴;

5 R⁸ is -CH₂C₆H₅, -CH₂C₆H₁₁, -CH₂C₅H₉, or -(CH₂)₃NHC(=NH)NH₂; and

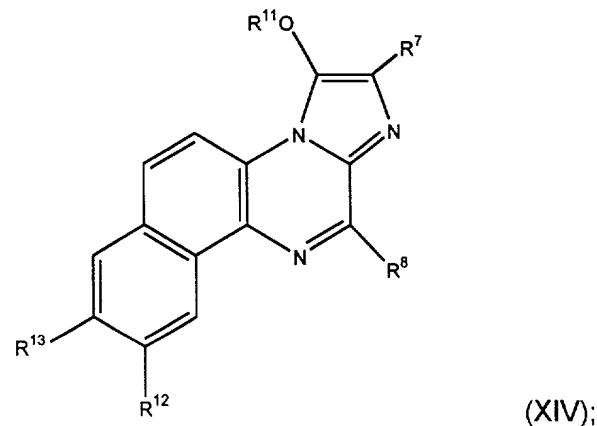
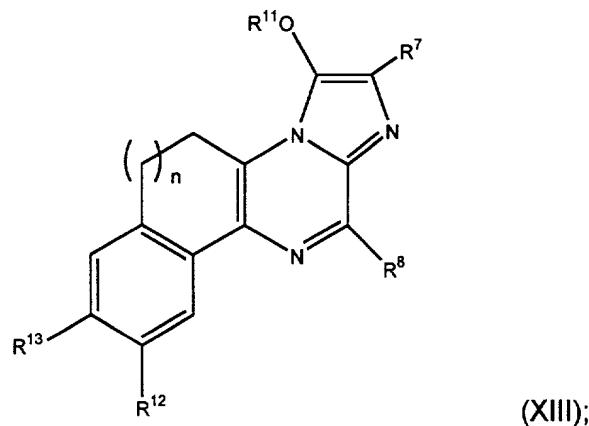
R⁹ is phenyl, indolyl, -C₆H₄OH, -C₆H₄NH₂, -C₆H₄F, or -C₆H₄OR¹⁵.

43. The method of claim 41, wherein R¹¹, R¹⁴, and R¹⁵ are esters.

44. The method of claim 41, wherein R¹¹, R¹⁴, and R¹⁵ are independently acetyl, butyryl, acetoxymethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl,

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45. The method of claim 39, wherein the protected luminophore is a compound of formula (XIII) or (XIV)



15

wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R¹² and R¹³ are independently -H, -OH, alkyl, heteroalkyl, aryl, or -OR¹⁶;

n is 0, 1, or 2; and

5 R¹¹, R¹⁴, and R¹⁶ are independently enzyme-removable groups.

46. The method of claim 45, wherein

R⁷ is -CH₂-C₆H₅, naphthyl, -CH₂-C₆H₄OH, -CH₂-C₆H₄F, or -CH₂-C₆H₄OR¹⁴; and

R⁸ is -CH₂C₆H₅, -CH₂C₆H₁₁, -CH₂C₅H₉, or -

10 (CH₂)₃NHC(=NH)NH₂.

47. The method of claim 45, wherein R¹¹, R¹⁴, and R¹⁶ are esters.

48. The method of claim 45, wherein R¹¹, R¹⁴, and R¹⁶ are independently acetyl, butyryl, acetoxyethyl, propanoyloxymethyl, butyryloxymethyl, or pivaloyloxymethyl.

15 49. The method of claim 45, wherein n is 1.

50. The method of claim 39, wherein the composition comprises a cell.

51. The method of claim 39, wherein the composition comprises a cell which contains the deprotecting enzyme.

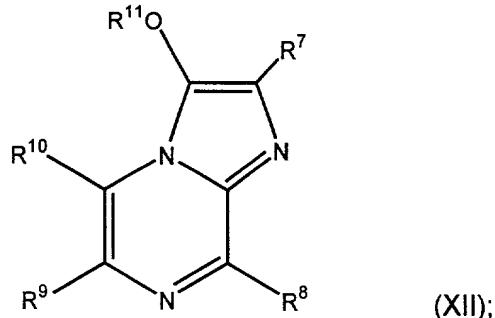
20 52. The method of claim 51, wherein detecting light produced from the composition indicates the location of the deprotecting enzyme in a cell.

53. The method of claim 39, wherein the composition comprises a cell lysate.

25 54. The method of claim 39, wherein the deprotecting enzyme is an esterase.

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55. The method of claim 39, wherein the solution is an aqueous solution.
56. The method of claim 39, wherein the solution comprises DMSO.
57. The method of claim 39, wherein the protected luminophore is a modified coelenterazine;
- wherein the enol group has been converted to an ester or an ether comprising an enzyme-removable group.
58. A method of generating luminescence in a living cell comprising a luciferase, the method comprising:
- 10 contacting the cell in solution with a protected luminophore.
59. The method of claim 58, wherein the protected luminophore is a modified coelenterazine;
- wherein the enol group has been converted to an ester or an ether comprising an enzyme-removable group.
- 15 60. The method of claim 58, wherein the protected luminophore is a compound of formula (XII)



wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

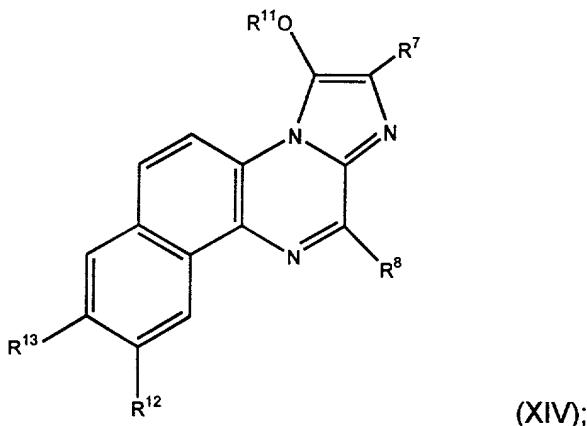
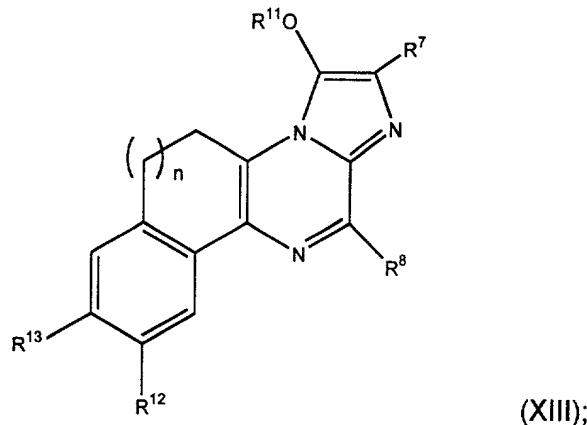
R⁸ is H, alkyl, heteroalkyl, or aryl;

R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

R¹⁰ is -H, -CH₃, or -CH(CH₃)₂; and

R¹¹, R¹⁴, and R¹⁵ are independently enzyme-removable groups.

61. The method of claim 58, wherein the protected luminophore is a compound of formula (XIII) or (XIV)



5

wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R¹² and R¹³ are independently -H, -OH, alkyl, heteroalkyl, aryl, or -OR¹⁶;

n is 0, 1, or 2; and

10 R¹¹, R¹⁴, and R¹⁶ are independently enzyme-removable groups.

62. A method of measuring the enzymatic activity of a non-luminogenic enzyme, comprising:

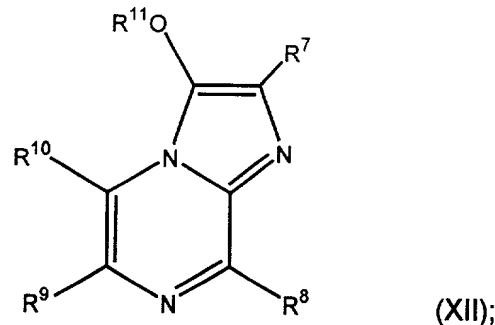
15 contacting a non-luminogenic enzyme with a liquid mixture comprising a luminogenic protein and a protected luminophore to form a composition; and

detecting light produced from the composition.

63. The method of claim 62, wherein the protected luminophore is a modified coelenterazine;

wherein the enol group has been converted to an ester or an ether comprising a group that is removable by the non-luminogenic enzyme.

5 64. The method of claim 62, wherein the protected luminophore is a compound of formula (XII)



wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

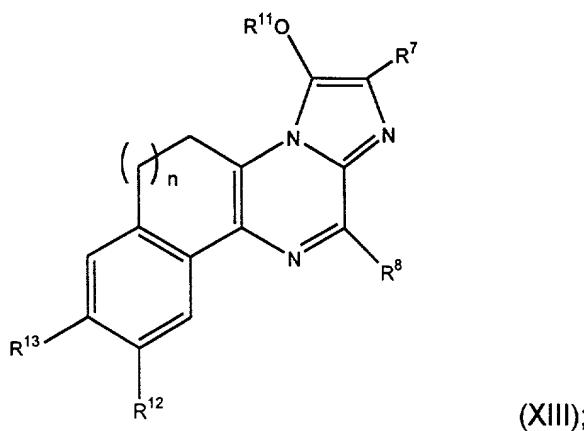
R⁸ is H, alkyl, heteroalkyl, or aryl;

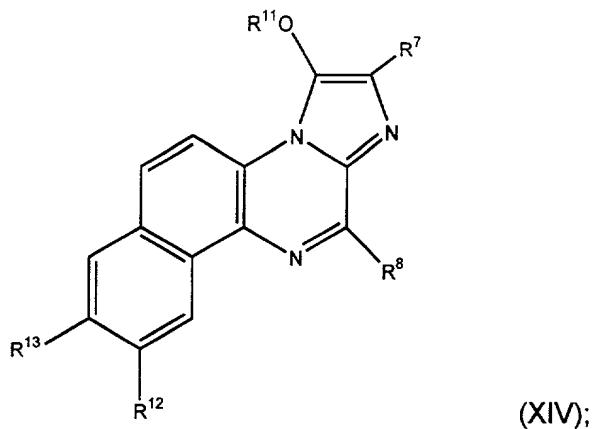
R⁹ is H, alkyl, heteroalkyl, aryl, or -C₆H₄OR¹⁵;

R¹⁰ is -H, -CH₃, or -CH(CH₃)₂; and

10 R¹¹, R¹⁴, and R¹⁵ are independently enzyme-removable groups that are removable by the non-luminogenic enzyme.

15 65. The method of claim 62, wherein the protected luminophore is a compound of formula (XIII) or (XIV)





wherein R⁷ is H, alkyl, heteroalkyl, aryl, or -CH₂-C₆H₄OR¹⁴;

R⁸ is H, alkyl, heteroalkyl, or aryl;

R¹² and R¹³ are independently -H, -OH, alkyl, heteroalkyl, aryl,

5 or -OR¹⁶;

n is 0, 1, or 2; and

R¹¹, R¹⁴, and R¹⁶ are independently enzyme-removable groups

that are removable by the non-luminogenic enzyme.